

# Heat stress abatement during the dry period influences prolactin signaling in lymphocytes

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## Abstract

Heat stress perturbs prolactin (PRL) release and affects dairy cow lactational performance and immune cell function. We hypothesized that greater PRL concentration in plasma of heat-stressed cows relative to cooled cows would decrease expression of prolactin receptor (*PRL-R*) mRNA and increase mRNA expression of suppressors of cytokine signaling (*SOCS*) in lymphocytes, altering their cytokine production. To test this hypothesis, multiparous Holstein cows were dried off 46 d before their expected calving date and assigned randomly to heat stress (HT;  $n = 9$ ) or cooling (CL;  $n = 7$ ) during the entire dry period. A second study was conducted the following year with an additional 21 cows (12 HT; 9 CL). Lymphocytes were isolated from cows at -46, -20, +2, and +20 d relative to expected calving date and mRNA expression of *PRL-R*, *SOCS-1*, *SOCS-2*, *SOCS-3*, cytokine-inducible SH2-containing protein (*CIS*), and heat shock protein 70 KDa A5 (*HSPA5*), and housekeeping genes hydroxymethylbilane synthase (*HMBS*), ATP synthase, H<sup>+</sup> transporting mitochondrial F1 complex, beta subunit (*ATP5B*), and ribosomal protein S9 (*RPS9*) was analyzed by quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR). Cows exposed to HT had greater PRL concentration in plasma compared with CL cows. Measurement of lymphocyte proliferation indicated that lymphocytes of CL cows proliferated more than those from HT cows and expressed more *PRL-R* mRNA and less *SOCS-1* and *SOCS-3* mRNA relative to HT cows. Further, lymphocytes from CL cows produced more tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) than those from HT cows. These results suggest that changes in PRL-signaling pathway genes during heat stress are associated with differential cytokine secretion by lymphocytes and may regulate lymphocyte proliferation in dairy cows.

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**Keywords:** Gene expression; Heat stress; Lymphocytes; Prolactin

## 1. Introduction

Prolactin (PRL) is known to regulate several physiological functions via its effects on cellular processes such as proliferation, differentiation, cell survival, and immune function [1]. The actions of PRL are mediated through the prolactin receptor (PRL-R). Auchtung et al. [2] reported that bovine lymphocytes express *PRL-R* mRNA and demonstrated through photoperiodic manipulations of calves that increased *PRL-R* expression is associated with lower circulating PRL concentrations

**Abbreviations:** CIS, cytokine-inducible SH2-containing protein; HSPA5, heat shock protein 70 KDa A5; JAK, Janus-associated kinase; LDPP, long-day photoperiod; PRL, prolactin; PRL-R, prolactin receptor; SDPP, short-day photoperiod; SOCS, suppressors of cytokine signaling; STAT, signal transducers and activators of transcription.

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and greater lymphocyte proliferation during a short-day photoperiod (SDPP) relative to a long-day photoperiod (LDPP). In addition, lymphocytes from steers exposed to an SDPP exhibited greater proliferation when incubated with PRL than lymphocytes from steers on an LDPP [3]. These results suggest that the observed increase in *PRL-R* mRNA in lymphocytes of SDPP steers results in enhanced effects of PRL on lymphocyte proliferation and potentially improved cellular immune function. Based on these findings, it was hypothesized [4] that negative effects of heat stress could be mediated through perturbed PRL signaling because heat stress is known to increase PRL secretion [5,6] and may affect immune function. However, the reported effects of heat stress on lymphocyte proliferation are conflicting, likely owing to differences among age and breeds of cattle evaluated [7–9], the intensity and duration of heat stress treatment [10,11], and variation in methodologies used among studies. For example, lymphocytes isolated from heifers of 3 beef breeds and exposed to 42 °C for 12 h had lower proliferation in response to phytohemagglutinin than those exposed to 38.5 °C [9]. In contrast, there was no effect of in vivo heat stress on stimulated proliferation of lymphocytes isolated from Holstein cows tested in vitro at 38.5 °C or 42 °C [8]. However, decreases in proliferation measured at 42 °C were reduced in cells obtained from heat-stressed cows, possibly because of an adaptation of the lymphocytes to high temperatures. It was further reported that lymphocytes isolated from Brown Swiss cows declined in proliferation at 41 °C, whereas those from Holstein cows declined at 42 °C [10]. In contrast, moderate heat stress did not affect lymphocyte proliferation [11], but high environmental temperatures were detrimental to lymphocyte proliferation, as measured by DNA synthesis [12]. Despite some controversy, it seems that heat stress impairs lymphocyte function. To date, the effects of heat stress during the entire dry period on cellular immune function of periparturient dairy cows has not been investigated.

The mechanism whereby heat stress affects immune status may be mediated through changes in the PRL-signaling pathway. Suppressors of cytokine signaling (SOCS) proteins and cytokine-inducible SH2-containing proteins (CIS) compose a family of intracellular proteins [13] that are stimulated by PRL, act through feedback to inhibit cytokine signaling [14], and regulate the responses of immune cells to cytokines [13]. In particular, SOCS-1 and SOCS-3 have been shown to bind to cytokine receptors or to receptor-associated Janus-associated kinases (JAK) to inhibit activation of signal transducers and activators of transcription (STAT) members, and ultimately interferon

signaling [15,16]. The SOCS proteins also regulate tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-mediated cellular apoptosis by inhibiting phosphatidylinositol 3-kinase and p38 mitogen-activated protein kinase pathways [17,18]. With changes in PRL concentration in plasma, it is plausible that effects of heat stress on immune function may be mediated through the PRL-signaling pathway and lymphocyte cytokine production. Specifically, we hypothesize that greater circulating PRL concentrations in plasma of heat-stressed cows would decrease expression of *PRL-R* mRNA in lymphocytes and increase mRNA expression of SOCS proteins, ultimately affecting cytokine production. Our objectives were to evaluate lymphocyte proliferation, cytokine production, and mRNA expression of *PRL-R* and *SOCS-1*, *SOCS-2*, *SOCS-3*, and *CIS* in lymphocytes isolated from heat-stressed versus cooled dairy cows during the dry period.

## 2. Materials and methods

### 2.1. Animals, housing, and sampling

The experiments were conducted at the University of Florida Dairy Research Unit in Hague, FL. All experimental animals were managed according to the guidelines approved by the University of Florida IFAS Animal Research Committee.

In the initial study, a subset of cows ( $n=4$  per treatment) from a larger study [19] were used as blood donors to assay lymphocyte proliferation. Briefly, 16 multiparous Holstein cows were assigned randomly to treatment after blocking by mature equivalent milk production. Treatments were imposed at dry off (46 d prior to expected calving date), continued until calving, and included heat stress (HT;  $n=9$ ) or cooling (CL;  $n=7$ ). The cooling system consisted of fans and sprinklers that turned on automatically when the ambient temperature exceeded 21.1 °C, which is the temperature at which heat stress begins in a humid environment such as Florida. After calving, all cows were housed together in a sand-bedded, free-stall barn equipped with fans and sprinklers. In the next year, the study was repeated with 21 animals (HT = 12 and CL = 9) to more fully characterize gene expression under heat stress conditions.

Blood (10 mL) was collected at 7:00 AM at -46, -32, -18, and -7 d relative to calving from coccygeal vessels into sodium-heparinized tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed on ice until centrifuged at  $2600 \times g$  at 5 °C for 30 min. Plasma was separated and frozen at -20 °C

for subsequent prolactin analysis by radioimmunoassay (RIA) [2].

For lymphocyte proliferation and total RNA extraction, 30 mL of blood was collected from a coccygeal vessel of each cow using heparinized tubes (Vacutainer, Becton Dickinson) -46, -20, +2 and +20 d relative to calving, and the -46 d sample was considered as the baseline, whereas only the +2 and +20 samples were analyzed for lymphocyte production of cytokines.

## 2.2. Lymphocyte isolation, proliferation, production of cytokines, and RNA extraction

Lymphocytes were isolated and stimulated with concanavalin A (ConA) to produce cytokines (TNF- $\alpha$ , IL-4, IL-6, and IFN- $\gamma$ ) or stored in Trizol (Invitrogen, Carlsbad, CA) at -80 °C until RNA extraction. Samples were transported to the laboratory at ambient temperature, and isolation of lymphocytes was initiated within 3 h of blood collection. Tubes were centrifuged at  $1000 \times g$  for 30 min at room temperature. The buffy coat was transferred to a tube containing 2 mL of medium 199 (M-199; Sigma-Aldrich, Saint Louis, MO) and gently mixed. The cell suspension was layered on 2 mL of Fico/Lite LymphoH (Atlanta Biologicals, Lawrenceville, GA) and centrifuged at  $250 \times g$  for 30 min at room temperature. Mononuclear cells were collected from the Fico/Lite interface and transferred to culture tubes containing 2 mL of Red Blood Cell Lysing Buffer (Sigma-Aldrich). Exactly 20 sec after transferring, the solution was neutralized with 8 mL of 1X DPBS (Sigma-Aldrich) and centrifuged at  $250 \times g$  for 15 min at room temperature.

The pellet (mononuclear cells) was washed once in 2 mL of M-199. The final pellet was resuspended in M-199 supplemented with 5% horse serum, 500 U/mL penicillin, 0.2 mg/mL streptomycin, 2 mM glutamine, and  $10^{-5}$  M  $\beta$ -mercaptoethanol. Viable lymphocytes were counted using the Trypan blue dye exclusion method. The cell suspension was adjusted to  $1 \times 10^6$  cells/mL for the proliferation assay or  $2 \times 10^6$  cells/mL for the cytokine production assay. Cell suspensions were plated in triplicate (lymphocyte proliferation; 20  $\mu$ L/well) or duplicate (cytokine production; 1 mL/well) with modified M-199 media (M-199 supplemented with 5% horse serum, 500 U/mL penicillin, 0.2 mg/mL streptomycin, 2 mM glutamine,  $10^{-5}$  M  $\beta$ -mercaptoethanol; all reagents from Sigma-Aldrich, Saint Louis, MO) and stimulated or not stimulated with 10  $\mu$ g/mL of ConA (Sigma-Aldrich) in a 96-well plate (lymphocyte proliferation) or a 6-well plate (Corning Inc., Corning, NY) for production of the cytokines. Lymphocytes (100  $\mu$ L) were plated with 20  $\mu$ L of ConA or

phosphate buffer (for control) and 80  $\mu$ L of modified M-199 to a final volume of 200  $\mu$ L. The 96-well plate was incubated at 37 °C with 5% CO<sub>2</sub> for 48 h. After incubation, 0.1  $\mu$ Ci [<sup>3</sup>H]thymidine in 10  $\mu$ L of culture medium were added. Cells were harvested 24 h after [<sup>3</sup>H]thymidine addition onto fiberglass filters using a cell harvester (Brandel, Gaithersburg, MD). Filters were counted for radioactivity using scintillation spectrometry (Beckman Coulter, Inc., Fullerton, CA).

Percent proliferation was calculated as average counts for ConA wells divided by average counts for phosphate buffer wells times 100. Cytokines in the incubation media were analyzed by Thermo Fisher Scientific using the SearchLight proteome arrays (Aushon bioSystems, Billerica, MA). Briefly, samples were incubated for 1 h on the array plates that were prespotted with capture antibodies specific for each protein biomarker. Plates were decanted and washed 3 times before adding a cocktail of biotinylated detection antibodies to each well. After incubating with detection antibodies for 30 min, plates were washed 3 times and incubated for 30 min with streptavidin-horseradish peroxidase. All incubations were done at room temperature with shaking at 200 rpm. Plates were again washed before adding a chemiluminescent substrate. The plates were immediately imaged using the SearchLight imaging system, and data were analyzed using SearchLight Array Analyst software, version (Aushon bioSystems.).

## 2.3. RNA extraction and qRT-PCR

Total RNA was extracted from lymphocytes using the recommended method for isolation of RNA from cells (Qiagen Inc., Valencia, CA). Briefly, lymphocytes stored on Trizol were thawed and immediately extracted using chloroform and addition of an equal volume of 70% (v/v) ethanol to the aqueous phase. Total RNA was isolated using the RNeasy Mini kit and on-column DNase digestion (Qiagen, Inc.). Quality of RNA was determined using the Agilent 2100 Bioanalyzer with RNA 6000 Nano LabChip kits (Agilent Technologies, Palo Alto, CA), and RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE). The iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) was used for first-strand cDNA synthesis using 2  $\mu$ g of total RNA per 80- $\mu$ L reaction volume. A parallel negative control reaction was performed in the absence of reverse transcriptase enzyme. Reaction conditions were 25 °C for 5 min, 42 °C for 30 min, and 85 °C for 5 min.

Primers were designed based on bovine nucleotide sequences available in GenBank for each gene using

Table 1  
Primer sequences, annealing temperature and amplicon size of each gene target analyzed in bovine lymphocytes by quantitative real-time PCR

Target	Primer	Sequence (5' → 3')	Size (bp)	Temp (°C)	GenBank accession number
<i>PRL-R</i>	Forward	GAACCTCAGGCCCATCCCT	65	56	NM.174155
	Reverse	TCCGGATTCTCCAGCTTCTC			
<i>SOCS-1</i>	Forward	CACAGCAGAAAAATAAAGCCAGAGA	94	56.8	XM.864316
	Reverse	CTCGTACCTCCTACCTCTTCATGTT			
<i>SOCS-2</i>	Forward	GGGATGCTTCCCTTCTTAAG	145	60	NM.177523
	Reverse	GTGCTGGGACCTTTCACCTA			
<i>SOCS-3</i>	Forward	GGCCACTCTCCAACATCTCTGT	99	56.8	NM.174466
	Reverse	TCCAGGAACCTCCGAATGG			
<i>CIS</i>	Forward	TCACACGACTGGAGGTCAG	107	60	NM.001046586
	Reverse	CCACACGGCTGAGATAAGGT			
<i>HSPA5</i>	Forward	GCCCAAGTGGCTGTTTACTG	126	63.3	NM.001075148
	Reverse	AGGTGAACACACGCCCTAAC			
<i>HMBS</i>	Forward	TGCTTCCTCCTGGCTTCAC	127	58.2	NM.001046207
	Reverse	GTTCTTACCACACTTCTCTCTG			
<i>ATP5B</i>	Forward	CATCGTGGCGGTCATTGG	148	58.2	NM.175796
	Reverse	AATGGTCCTTACTGTGCTCTC			
<i>RPS9</i>	Forward	GCTGATCGGCGAGTATGG	148	55.7	NM.001101152
	Reverse	CCGCAACAGGGCATTACC			

Primer3 software (<http://frodo.wi.mit.edu/>). Transcript abundance for each gene was determined by absolute quantitative real-time polymerase chain reaction (PCR) using the Bio-Rad iCycler or MyiQ Real-Time PCR Detection System (Bio-Rad Laboratories). Reactions were performed in duplicate using 2  $\mu$ L of cDNA, 0.4  $\mu$ M of each primer, and 12.5  $\mu$ L of iQ SYBR Green Supermix (Bio-Rad Laboratories) in a 25- $\mu$ L reaction volume. Reaction cycling conditions were 95 °C for 3 min followed by 45 cycles of 94 °C for 15 sec, annealing temperature for 30 sec, and 72 °C for 30 sec, with fluorescence measurement during the extension step. Table 1 summarizes the primer sequences, annealing temperature, and amplicon size of each gene target.

Identity of amplification products was confirmed by direct sequencing of gel-purified PCR-amplification products (QIAquick Gel Extraction Kit, Qiagen, Inc.) using a CEQ8000 automated DNA sequencer and DTCS Quickstart Chemistry (Beckman Coulter, Fullerton, CA). Concentrations of amplicons were determined using the NanoDrop ND-1000 spectrophotometer and used to create calibration curves for each gene. Standards ranging from  $10^2$  to  $10^7$  molecules were analyzed in duplicate with each assay. A single negative control reaction was run for each experimental sample, and a single blank reaction using water as the template was included with each standard curve. Copy numbers of transcripts in each sample were automatically determined by interpolation from the standard curve using iQ or MyiQ software (Bio-Rad Laboratories). Expression of *HMBS*, *ATP5B*, and *RPS9* was evaluated for stability using GeNorm

(<http://medgen.ugent.be/%7Ejvdesomp/genorm/>), and *HMBS* was found to be stable over time and between treatments ( $M = 0.375$ ). Final transcript abundance was expressed as the number of molecules per unit of total RNA used in the reverse transcription reaction normalized to *HMBS*. The first lymphocyte sample taken at -46 d relative to calving (ie, dry off) was considered as the baseline, and all data were expressed relative to the baseline value.

#### 2.4. Statistical Analysis

Repeated-measures data were analyzed using PROC MIXED procedures of SAS, version (SAS Institute, Cary, NC). The model included the fixed effects of treatment, time, and treatment-by-time interaction and the random effect of cow. Data were tested to determine the

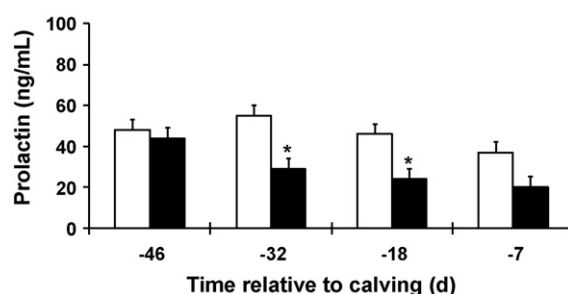


Fig. 1. Effect of cooling (CL;  $n = 7$ ) or heat stress (HT;  $n = 5$ ) during a 46-d dry period on prolactin concentration in plasma. The solid bar represents cows exposed to CL and the open bar represents cows under HT. \*  $P < 0.05$ .

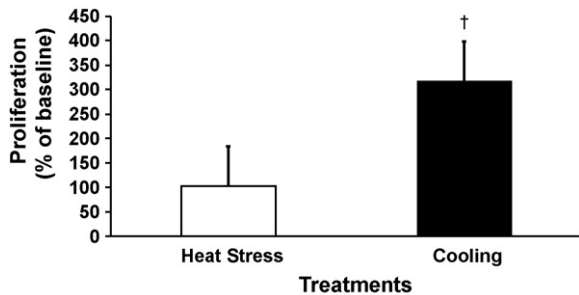


Fig. 2. Effect of cooling (CL;  $n=4$ ) or heat stress (HT;  $n=4$ ) during a 46-d dry period on lymphocyte proliferation in response to concanavalin A. The solid bar represents cows exposed to CL and the open bar represents cows under HT. Lymphocyte proliferation at -46 d relative to calving was considered as the baseline.  $\dagger P=0.08$ .

structure of best fit, namely AR (1), ARH (1), CS, or CSH, as indicated by a lower Schwarz Bayesian information criterion value [20]. Standard error of the mean is reported. For lymphocyte proliferation and cytokine production, all sampling dates were included in the model, and results are shown for main effect of treatment across time because no treatment-by-time interaction was detected. For lymphocyte gene expression, -46 d relative to calving was considered as the baseline, and treatment-by-time interaction was detected and figures are reported.

### 3. Results and discussion

#### 3.1. Prolactin concentration

As expected, plasma concentrations of PRL were similar between treatments at dry off, before treatments were imposed (Fig. 1). Once treatments were imposed, HT cows had greater PRL concentrations in plasma at -32 and -18 d relative to calving ( $P=0.05$ ; SEM = 5 ng/mL; Fig. 1). Similarly, other authors [5,6] reported that heat stress increases PRL concentrations in cattle. Because the major environmental stimuli associated with seasonal changes, namely, ambient temperature and daylight duration, vary in a parallel manner, it is plausible that some portion of seasonal shifts in immune status are driven by changes in PRL signaling.

#### 3.2. Lymphocyte proliferation, production of cytokines, and gene expression

In our initial examination of the effect of heat stress on immune status, lymphocytes isolated from cows exposed to CL had greater proliferation when stimulated with ConA compared with those from HT cows (Fig. 2), as reported by others [8,12], indicating that duration and

intensity of heat stress compromise immune function. Indeed, cows exposed to HT had greater rectal temperature than those exposed to CL [19], suggesting that heat stress affected cow body temperature, and consequently immune cell response. These results suggest that our model of heat stress was appropriate for subsequent evaluation of gene expression within immune cells.

Heat stress increases PRL concentrations in plasma [5,6]. Normally, elevated PRL concentration in plasma causes a down-regulation of its receptor [21,22]. Thus, as expected, lymphocytes isolated from CL cows had greater *PRL-R* mRNA expression compared with HT cows (Fig. 3a). Previous research indicated that steers exposed to SDPP had greater lymphocyte proliferation relative to those exposed to LDPP, which was associated with greater expression of *PRL-R* mRNA and a lower plasma concentration of PRL [2]. In addition, steers on SDPP had increased responsiveness of lymphocytes to PRL [3]. Thus, based on these findings, we would expect HT cows to have greater circulating concentrations of PRL, lower *PRL-R* mRNA expression by lymphocytes, and exhibit lower lymphocyte proliferation, which is generally consistent with our findings.

The intracellular proteins SOCS-1 and SOCS-3 inhibit signal transduction of type I and type II cytokine receptors (such as *PRL-R*), possibly through action at the level of receptors and JAKs; however, CIS and SOCS-2 act mainly by competition with STAT factors for recruitment to activated receptor complexes [23]. Lymphocytes from cows exposed to CL had lower expression of *SOCS-1* and *SOCS-3* mRNA (Fig. 3b and d). The protein SOCS-1 is an essential negative regulator for T-cell activation by dendritic cells and for maintaining immunological tolerance by restricting CD8+ T-cell proliferation. Thus, low expression of SOCS-1 may enhance JAK2 activity, thereby promoting cell proliferation [13]. This hypothesis agrees with our results, in which lymphocytes from CL cows proliferate more than those of HT cows and expressed less *SOCS-1* mRNA. However, there was no effect of heat stress on mRNA expression of *SOCS-2* ( $P=0.92$ ) or *CIS* ( $P=0.30$ ; Fig. 3c and e).

In addition, *HSPA5* mRNA, which encodes a member of the HSP70 protein family, was up-regulated in lymphocytes from HT cows at +20 d relative to calving compared to CL cows, however, expression was not affected during the dry period in which heat stress was imposed (Fig. 3f). Likewise, no effect of summer heat stress was reported in the expression of HSP70 in lymphocytes isolated from grazing beef cattle [24]. In vitro data suggest that HSP70 require temperatures above 42 °C for activation [25], which may explain the lack of a treatment effect on *HSPA5* mRNA during the



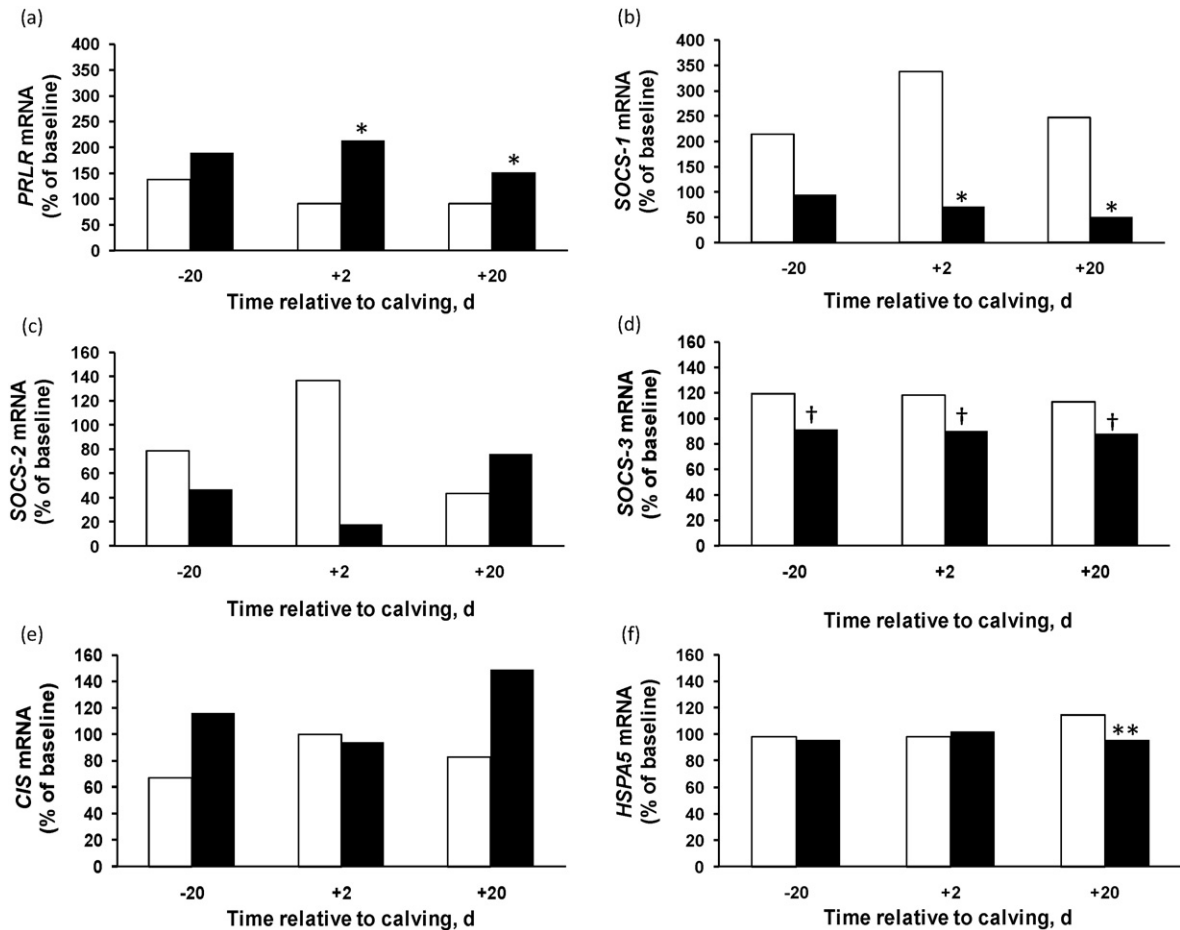


Fig. 3. Effect of cooling (CL;  $n = 7$ ) or heat stress (HT;  $n = 5$ ) during a 46-d dry period on the lymphocyte mRNA expression of *PRL-R* (a), *SOCS-1* (b), *SOCS-2* (c), *SOCS-3* (d), *CIS* (e), and *HSPA5* (f). Solid bar represents cows exposed to CL, and open bar represents cows exposed to HT. All genes of interest were normalized to *HMBS*. Gene expression at dry off was considered as the baseline. Lymphocytes isolated from cows exposed to CL had greater expression of *PRL-R* at +2 ( $P = 0.03$ ; SEM = 35%) and +20 ( $P = 0.05$ ; SEM = 20%) d relative to calving. Compared to lymphocytes from HT cows, those from CL cows had lower *SOCS-1* mRNA expression at +2 and +20 d relative to calving ( $P = 0.04$ ; SEM = 80%). There was no difference in *SOCS-2* mRNA expression between treatments ( $P = 0.58$ ; SEM = 49%). Compared to lymphocytes from HT cows, those from CL cows had lower *SOCS-3* mRNA expression ( $P = 0.09$ ; SEM = 13%). There was no difference in *CIS* mRNA expression between treatments ( $P = 0.30$ ; SEM = 45%). Lymphocytes isolated from cows exposed to CL had greater expression of *HSPA5* at +20 d relative to calving ( $P = 0.01$ ; SEM = 5%). \*\*  $P < 0.01$ . \*  $P < 0.05$ . †  $P < 0.10$ . Cows were housed in the same barn equipped with fans and sprinklers after parturition. Note that figures a-b and c-f are in the same scale.

dry period in the present study. However, there appeared to be a carry-over effect into lactation, indicating that the metabolic stress of lactation might also play a role in lymphocyte *HSPA5* mRNA expression. In fact, increased expression of HSP70 has been shown to be protective of cells to metabolic stress [26].

Lastly, in terms of cytokine production, lymphocytes isolated from HT cows also produced less TNF- $\alpha$  compared to those from CL cows (Fig. 4a). It has been reported that SOCS-1 inhibits TNF- $\alpha$  secretion [17,18]. In contrast, CIS can be induced by TNF- $\alpha$  [27]. Indeed, *CIS* mRNA expression in lymphocytes from CL cows

was numerically greater (120 vs 83%; SEM = 24%;  $P = 0.30$ ) than in those of HT cows (Fig. 3e). Unexpectedly, IFN- $\gamma$  and IL-4 secretion from the lymphocytes did not differ between treatments (Fig. 3b and c). We expected lower IFN- $\gamma$  and IL-4 production from the HT lymphocytes, as *SOCS-1* inhibits both IFN- $\gamma$  and IL-4 [23]. In addition, because SOCS-1 is stimulated by IL-6 [13], we expected greater secretion of IL-6 by lymphocytes from HT cows. However, IL-6 secretion did not differ between lymphocytes from HT and CL cows (Fig. 4d). The lack of response in IL-6 was possibly owing to metabolism of IL-6 early in the stimulation,

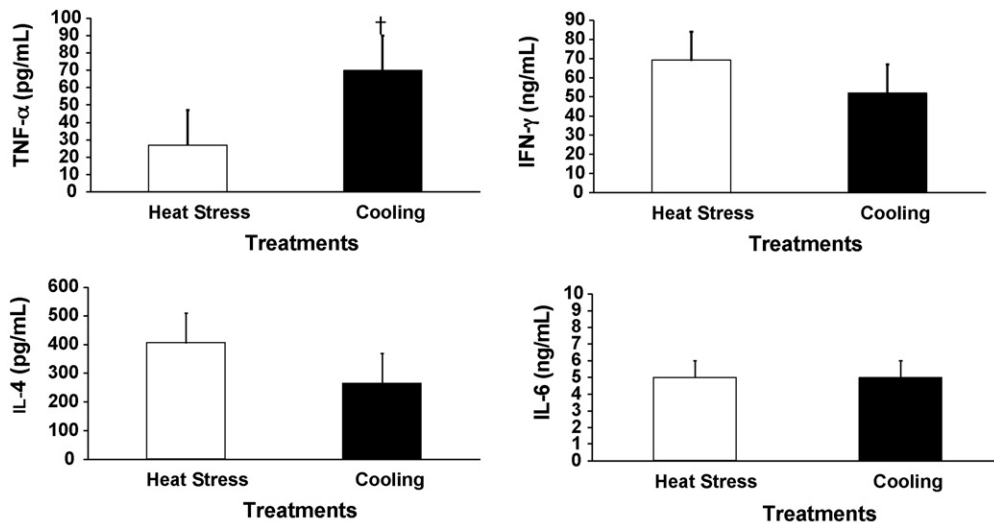


Fig. 4. Effect of cooling (CL;  $n = 7$ ) or heat stress (HT;  $n = 5$ ) during a 46-d dry period on the lymphocyte production of cytokines TNF- $\alpha$  (a), IFN- $\gamma$  (b), IL-4 (c), and IL-6 (d) on blood samples taken at +2 and +20 d relative to calving. Solid bars represent cows exposed to CL, and open bars represent cows exposed to HT. Lymphocytes from CL cows secreted more TNF- $\alpha$  compared to those from HT cows ( $\dagger P = 0.09$ ; SEM = 20 pg/mL). Secretion of IFN- $\gamma$  ( $P = 0.76$ ; SEM = 15 ng/mL), IL-4 ( $P = 0.82$ ; SEM = 104 pg/mL), and IL-6 ( $P = 0.59$ ; SEM = 1 ng/mL) did not differ between treatments. The main effect of treatment is reported since, no treatment-by-time interaction was detected. Cows were housed in the same barn equipped with fans and sprinklers after parturition.

such that the 48-h incubation period used to trigger an immune response did not allow us to detect the change over time. Indeed, IL-6 concentration was very low in the lymphocyte media, suggesting a high rate of metabolism in our culture system.

In summary, *PRL-R* and *SOCS* mRNA are differentially expressed between lymphocytes isolated from cows exposed to HT or CL. The greater *PRL-R* and lower *SOCS-1* and *SOCS-3* mRNA expressions in lymphocytes from CL cows were associated with greater lymphocyte proliferation and may be a mechanism whereby cooling cows improves immune cell function. In addition, the increased TNF- $\alpha$  cytokine production of lymphocytes of CL cows provides evidence of cell communication to mount an appropriate regulated immune response.

The observed responses of dry cows to heat stress are of interest from biological and practical perspectives. Coupled with previous reports on the impact of photoperiod manipulation on circulating PRL [2,3], the present results suggest that major environmental factors, namely, temperature and light, can act in parallel through PRL signaling to affect immune status. Because disease incidence is greater during the transition into lactation than at any other time in the lactation cycle [28,29], improved methods to manage the immune status of cows during this period would be advantageous to animal health, and ultimately to performance and well-being.

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